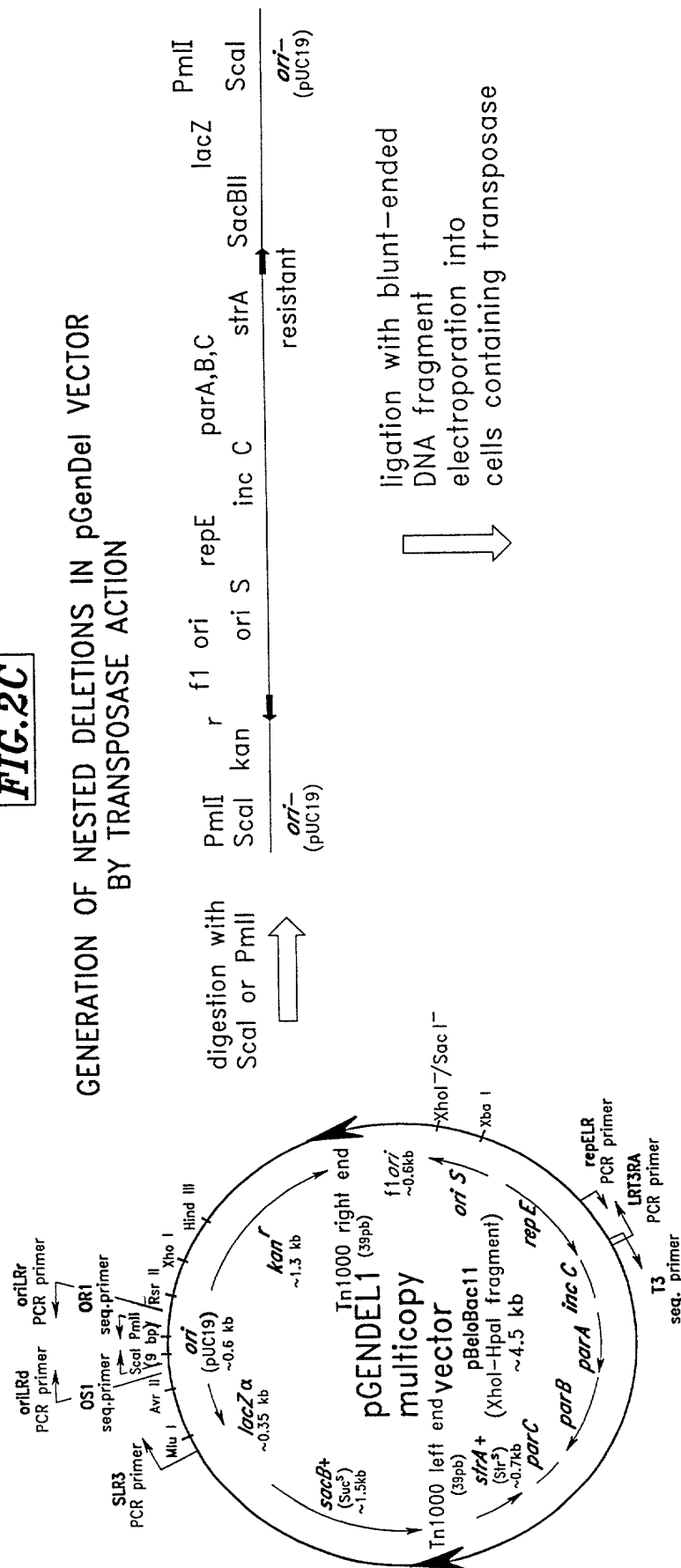


**FIG. 1**

**FIG. 2**

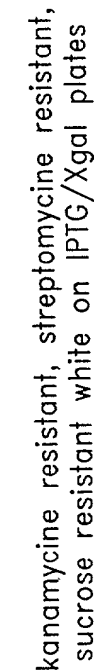
**FIG. 2A**  
**FIG. 2B**  
**FIG. 2C**

# GENERATION OF NESTED DELETIONS IN pGenDel VECTOR BY TRANSPOSASE ACTION



kanamycin resistant, streptomycin sensitive if introduced into streptomycin resistant host cells, sucrose sensitive deeply on IPTG/Xgal plates

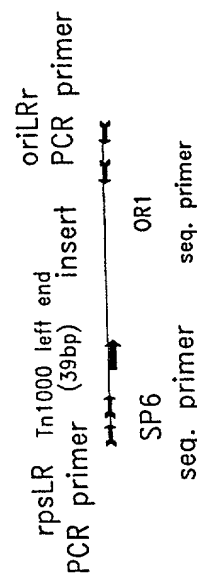
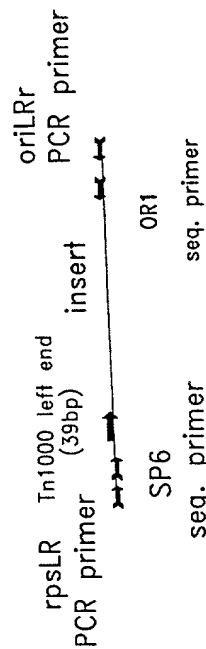
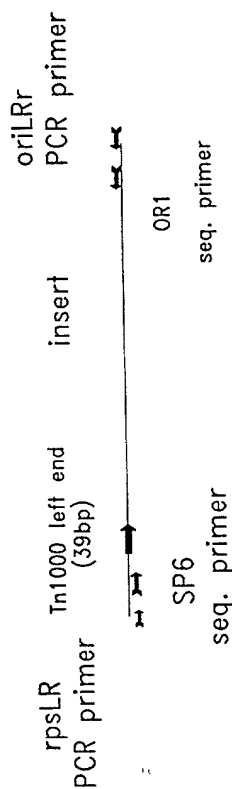
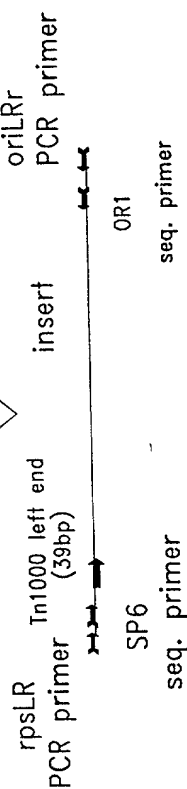
**FIG. 2A**



kanamycin resistant, streptomycin resistant if introduced into streptomycin resistant cells, sucrose sensitive faint blue on IPTG/Xgal plates

**FIG. 2B**

generation of templates  
by LR PCR  
from colonies



1. Forced cloning of blunt ended fragments into pGenDel by contra-selection on streptomycine plus kanaycine

2. Selection of intra transposed clones by plating on sucrose/kanamycine/Xgal media

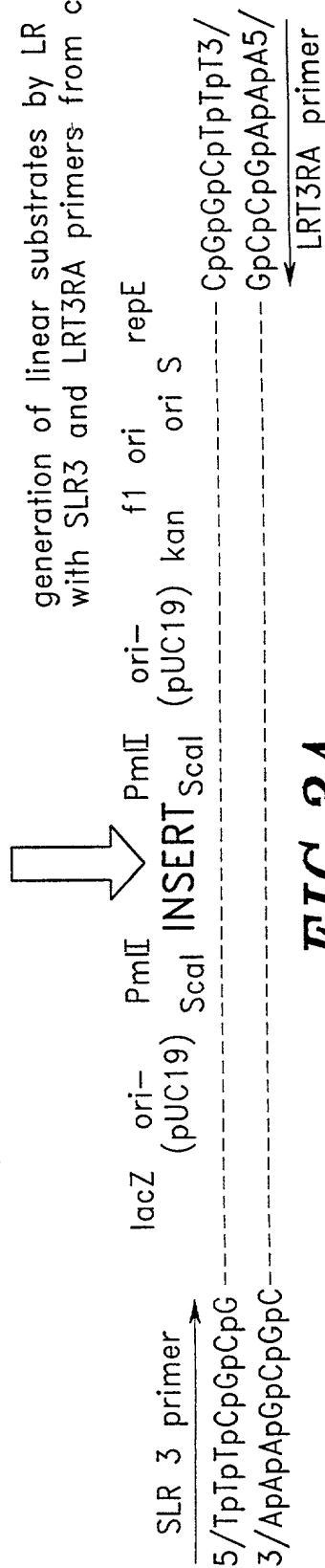
3. Generation of templates by PCR from colonies.

4. Minimal tiling path determination by sizing

**FIG.2C**



generation of linear substrates by LR PCR with SLR3 and LRT3RA primers from cells



**FIG. 3A**

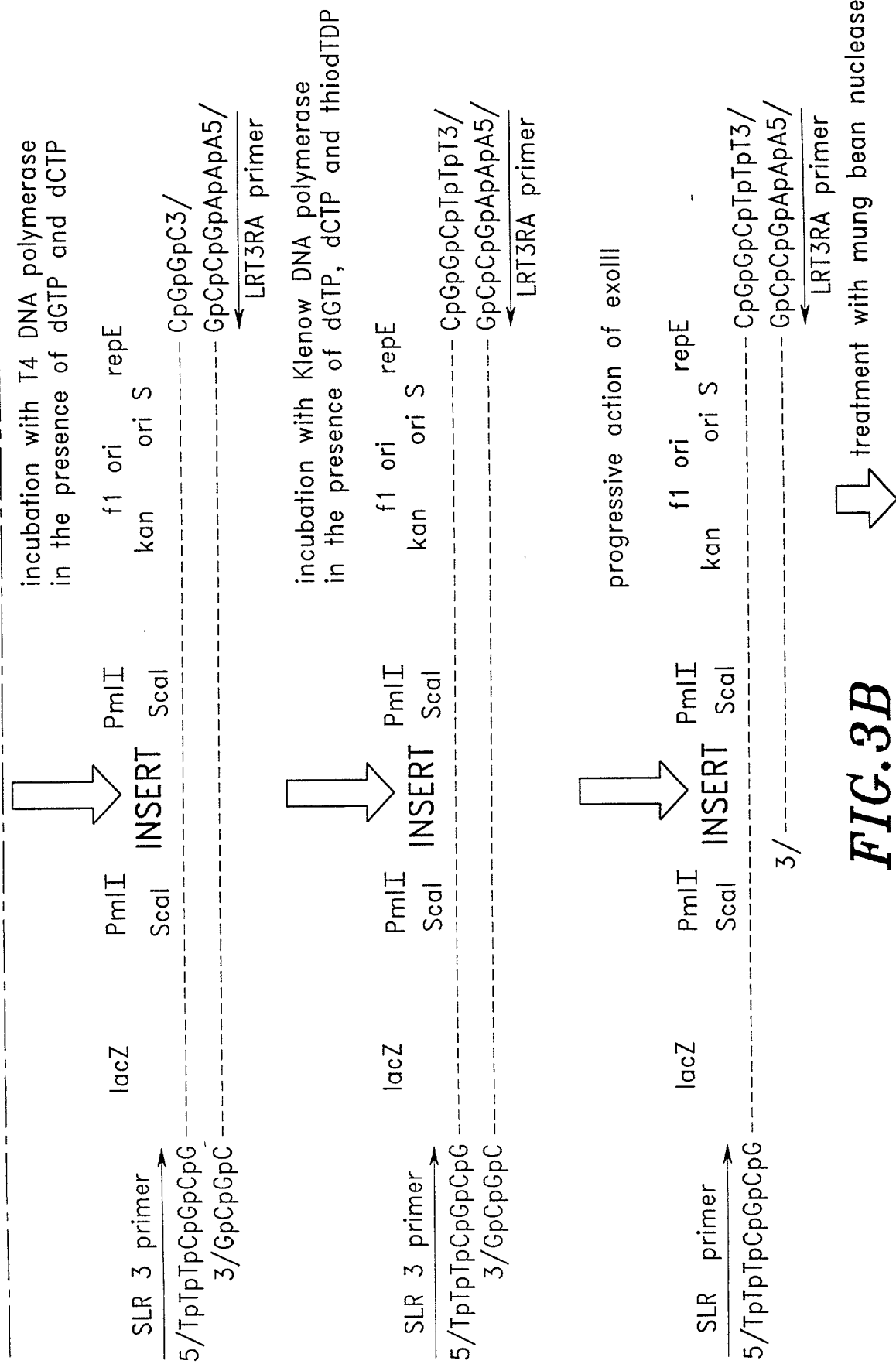
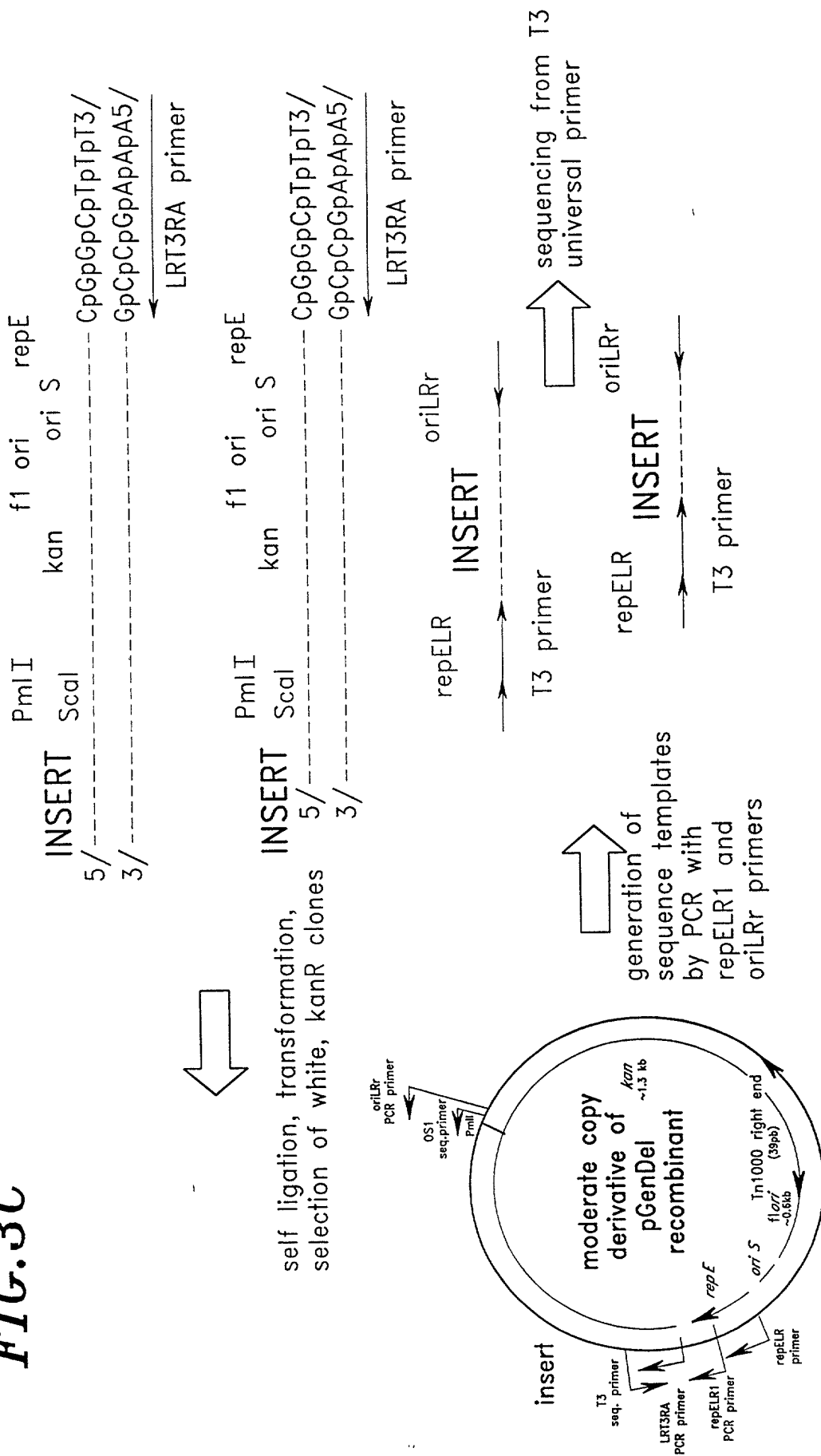


FIG. 3C

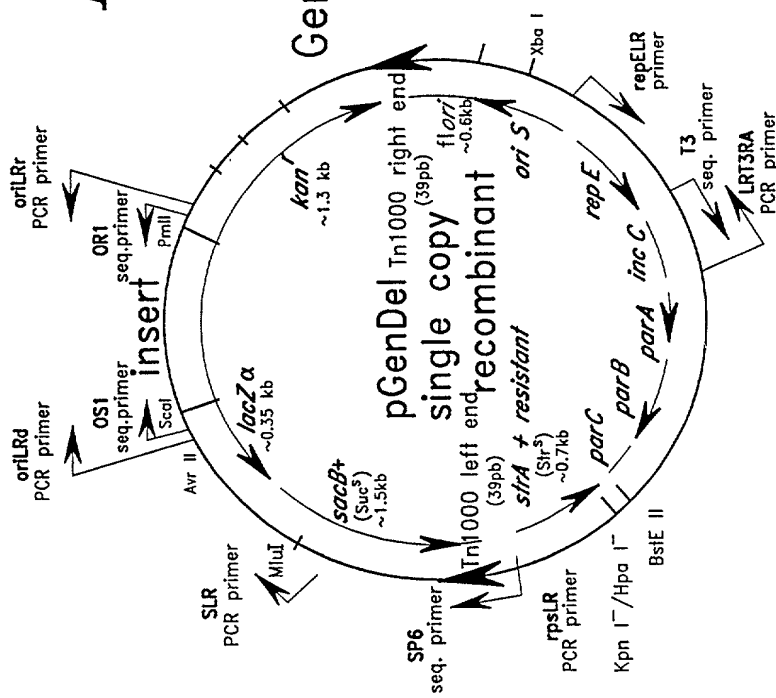


kanamycine resistant, streptomycine resistant if introduced into streptomycine resistant cells, sucrose sensitive, white on IPTG/Xgal plates

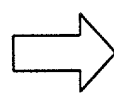
FIG. 4A  
FIG. 4B  
FIG. 4C

FIG. 4

Generation of nested deletions  
with Mung bean nuclease



kanamycin resistant, streptomycin resistant if introduced into streptomycin  
resistant cells, sucrose sensitive, faint blue on IPTG/Xgal plates



generation of linear substrates by LR PCR  
with SLR3 and LRT3RA primers from cells

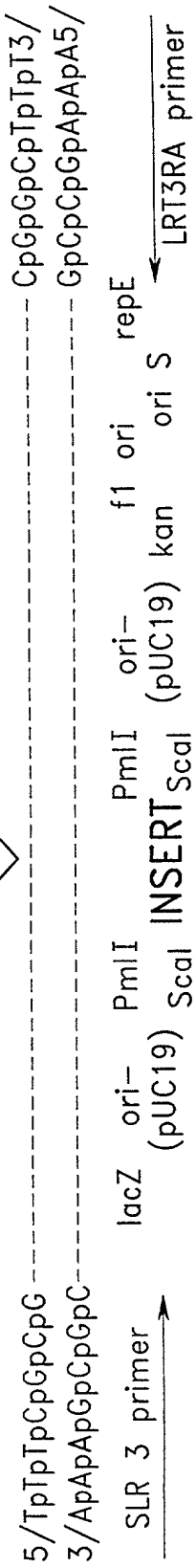
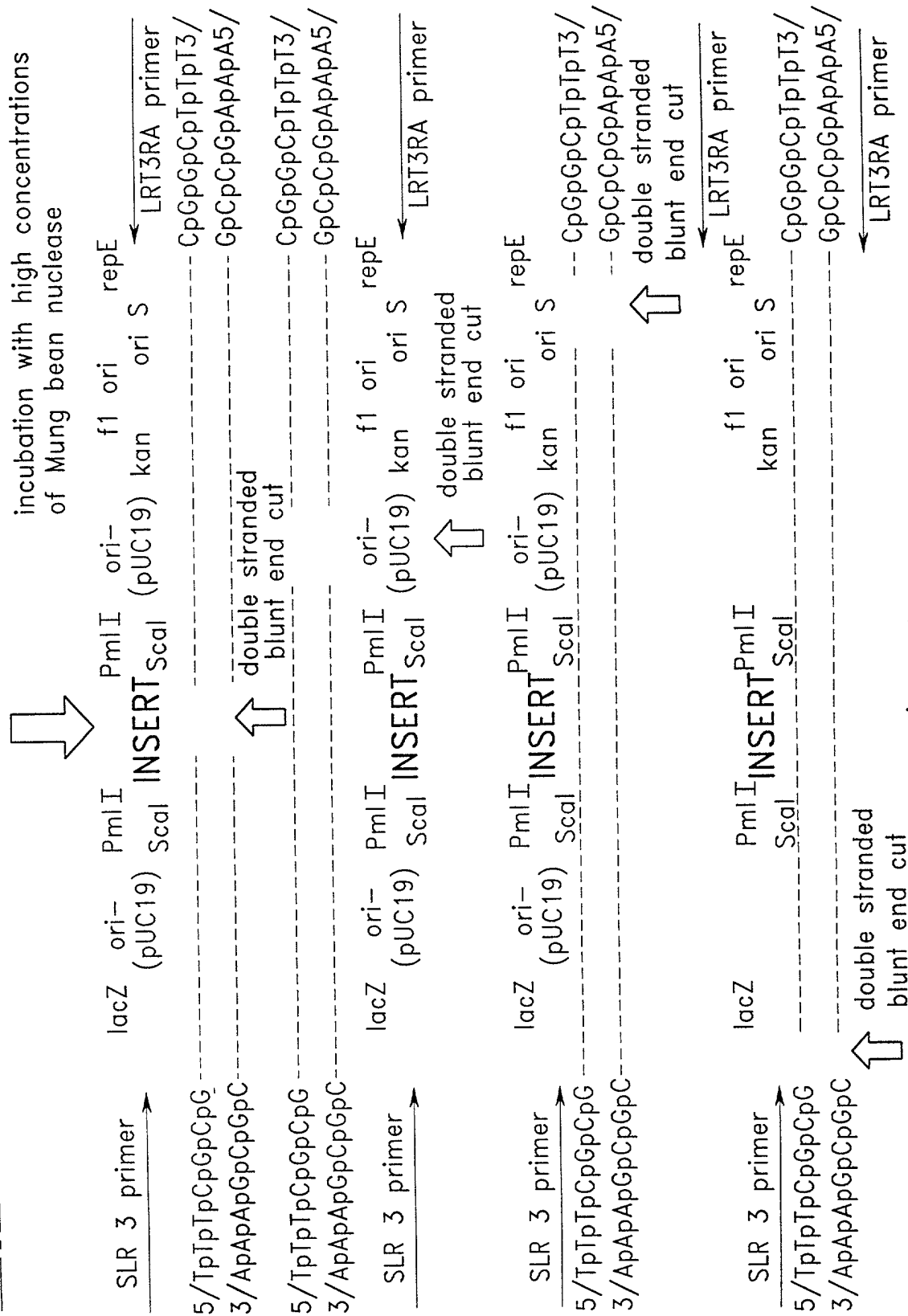
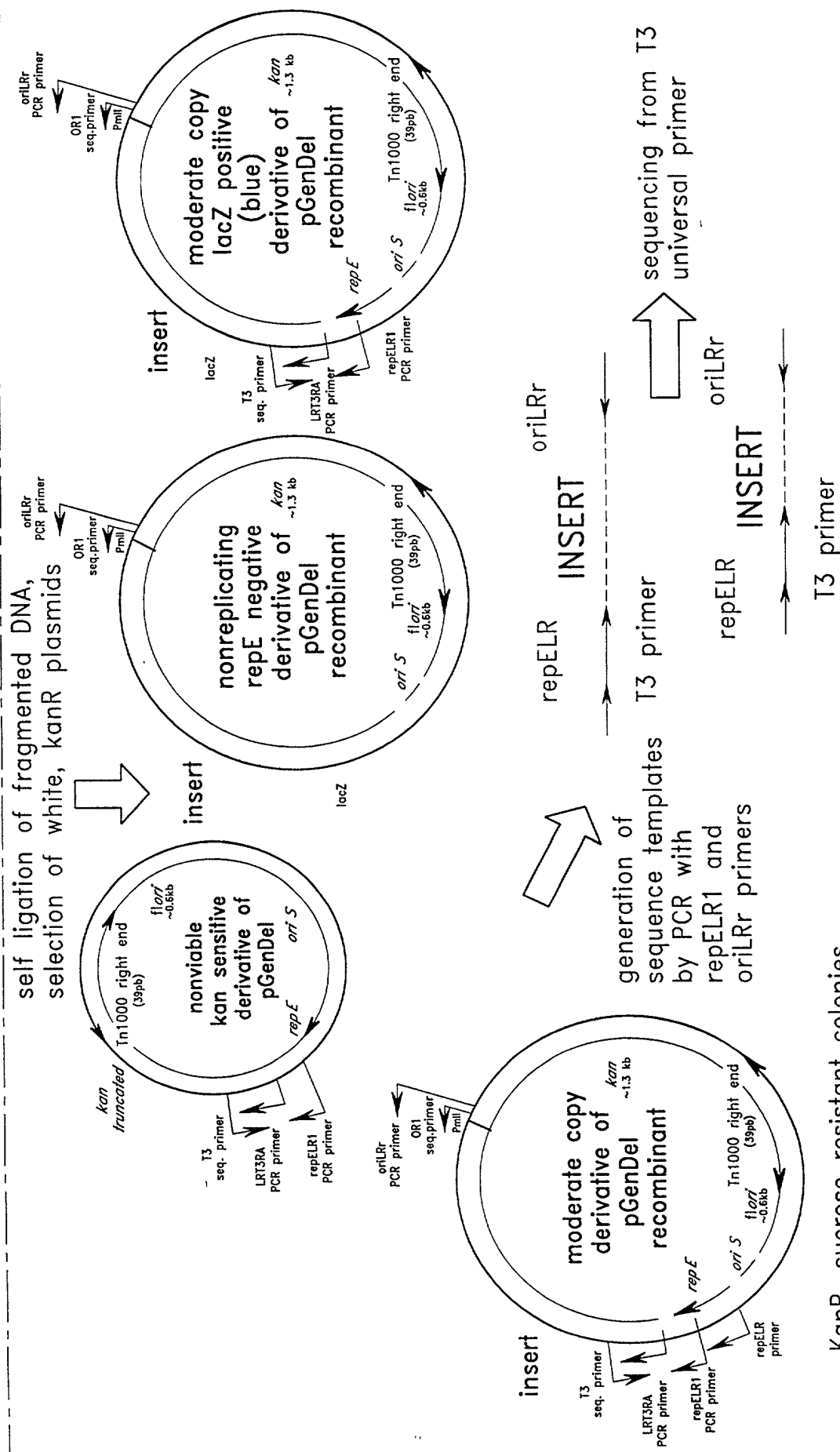


FIG. 4A





**FIG. 4B**

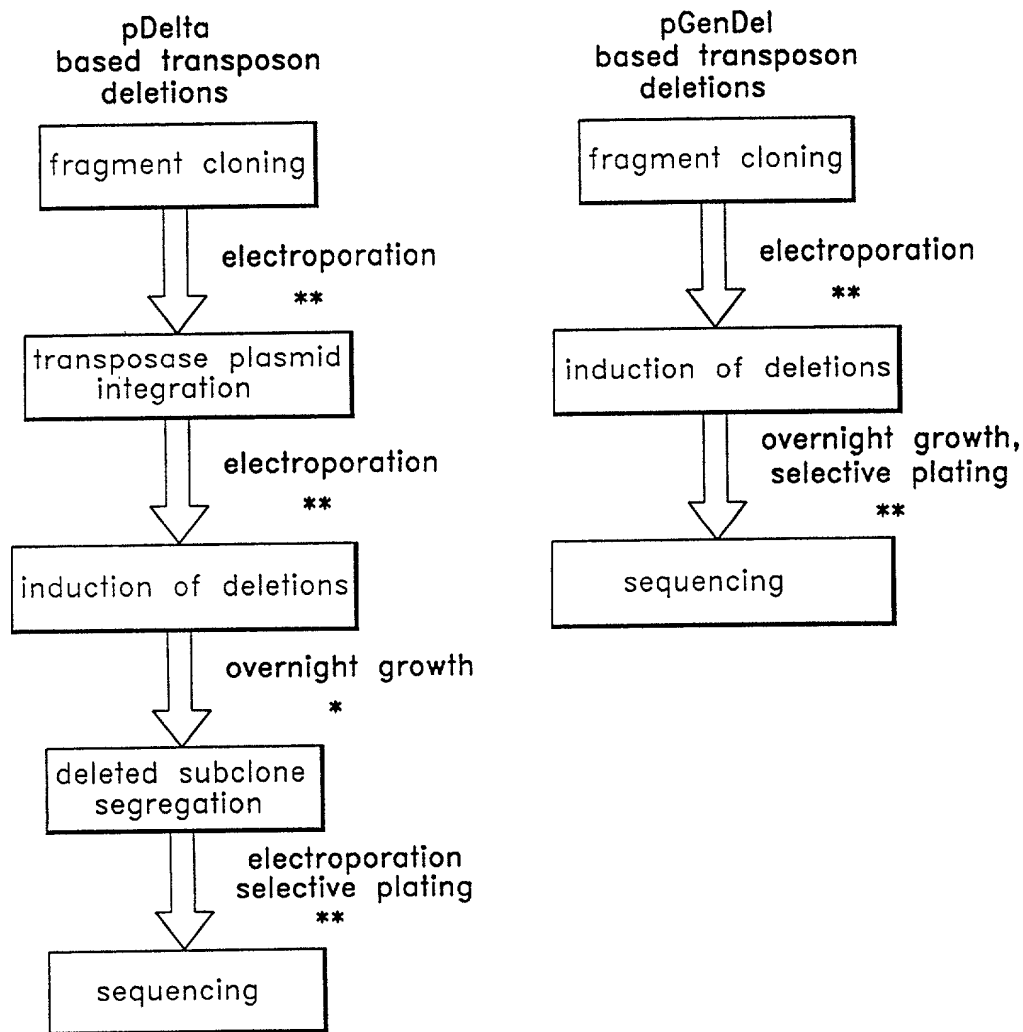


KanR, sucrose resistant colonies  
white on X-Gal IPTG

**FIG. 4C**

# FIG.5 FIG.5A FIG.5B

Comparison of different methods of  
nested deletion sequencing



\*shown in

\*— easy stages

\*\*— difficult stages

\*\*\*—very difficult stages

## FIG.5A

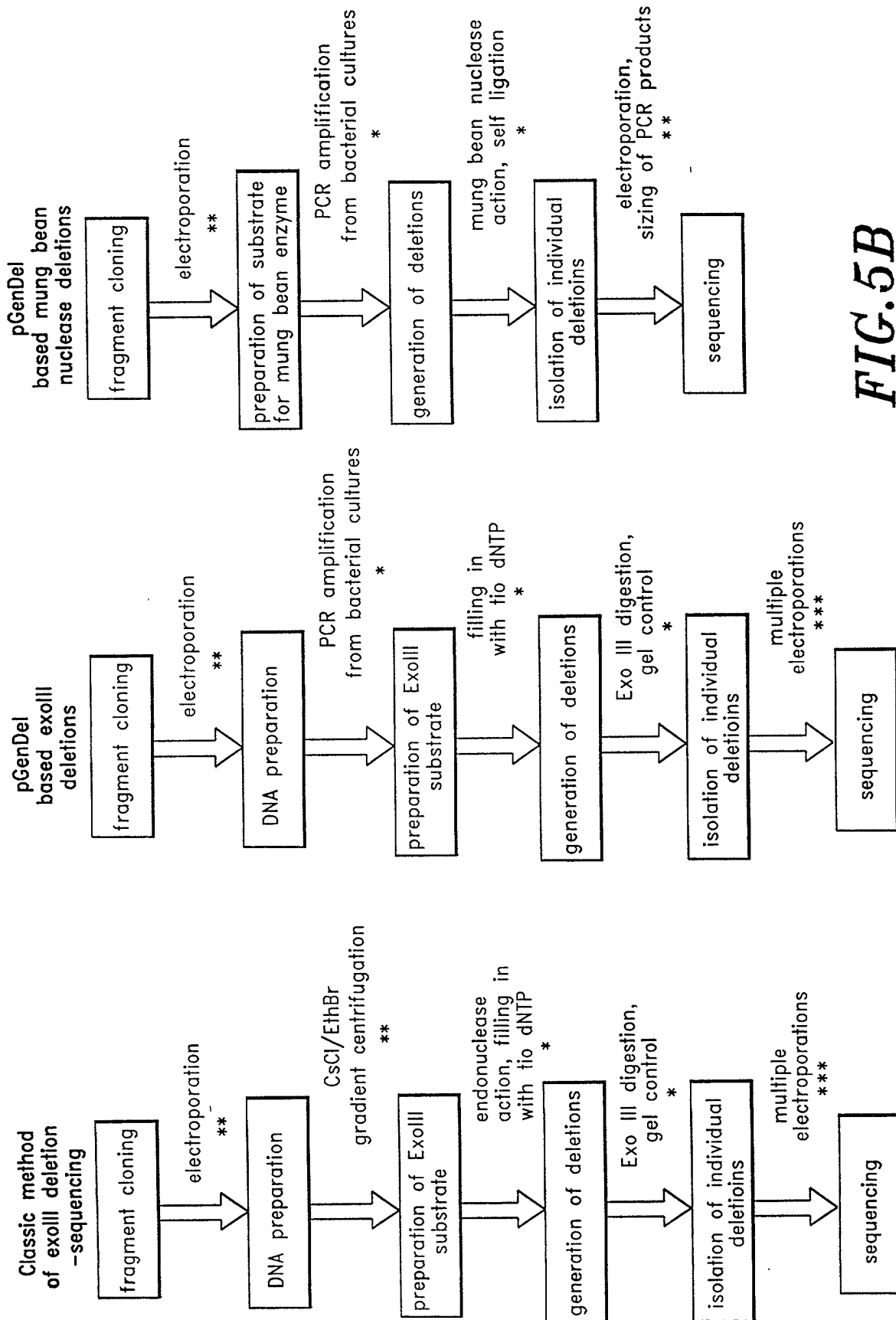


FIG.5B

# THE SHOTGUN STRATEGY

INSERT



SEQUENCES

_____	_____	_____
_____	_____	_____
=====	=====	=====

CONTIG1                      HOLE      CONTIG2

*FIG. 6*

FIG. 7 is a schematic diagram of the pairwise strategy for identifying subclones.

# THE PAIRWISE STRATEGY

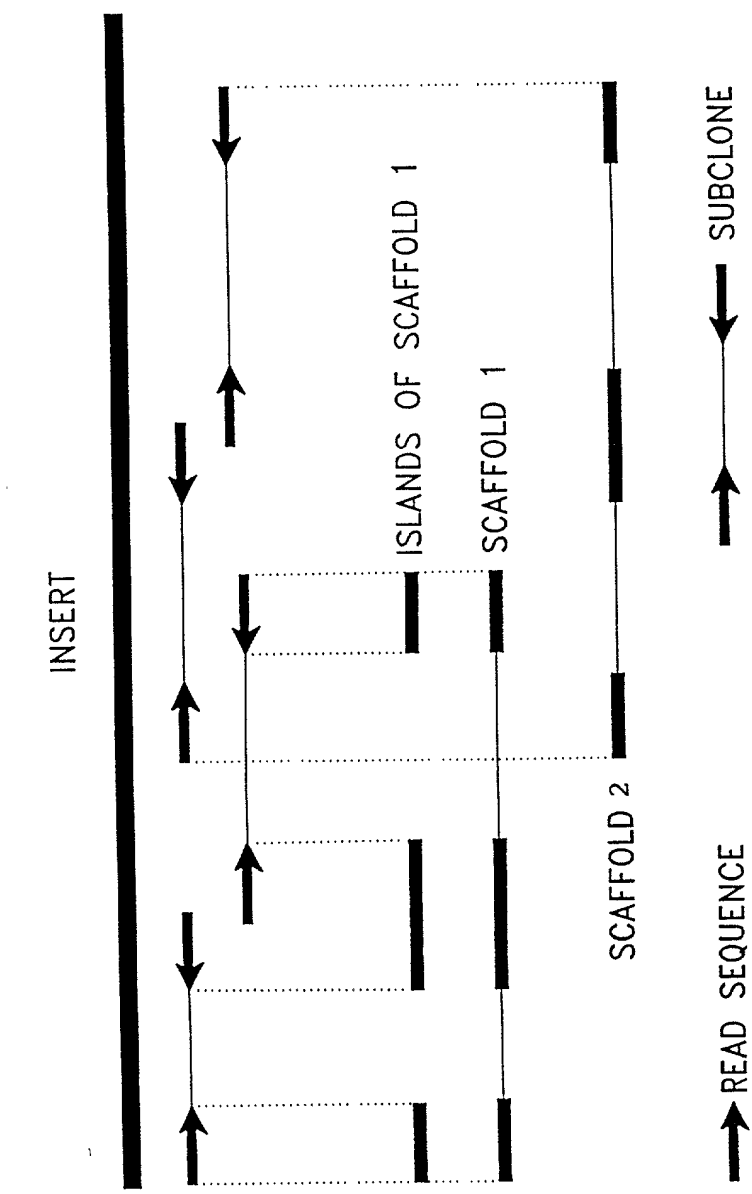


FIG. 7

# MULTIPLE NUCLEATION POINT

INSERT

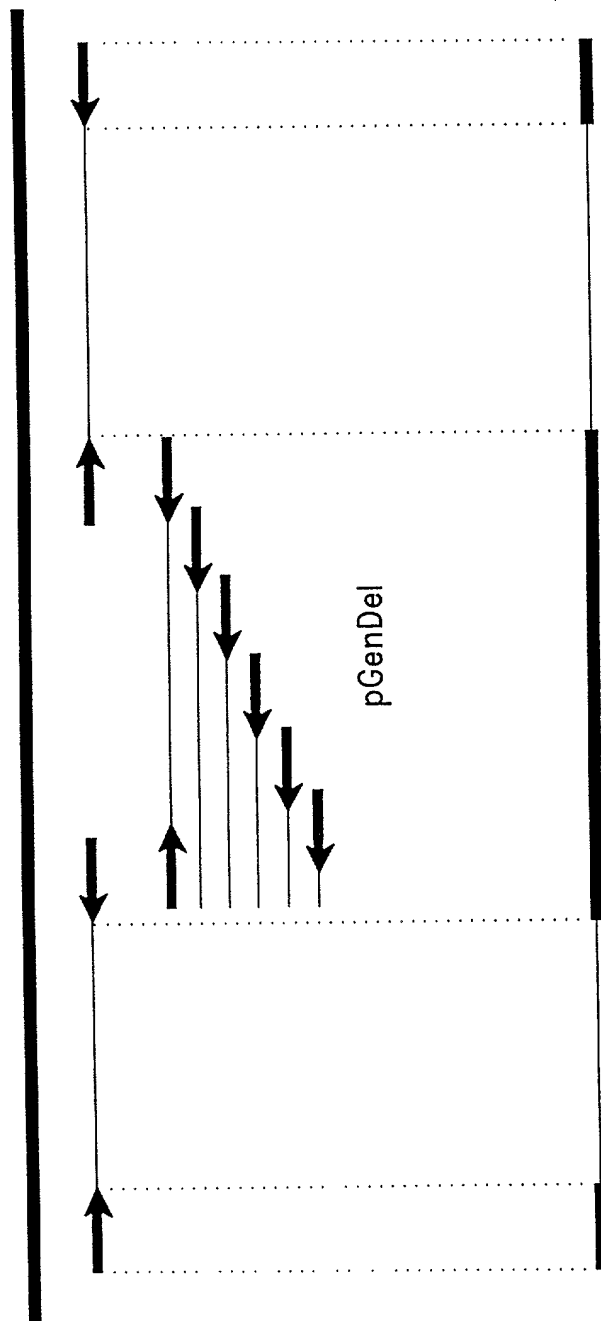
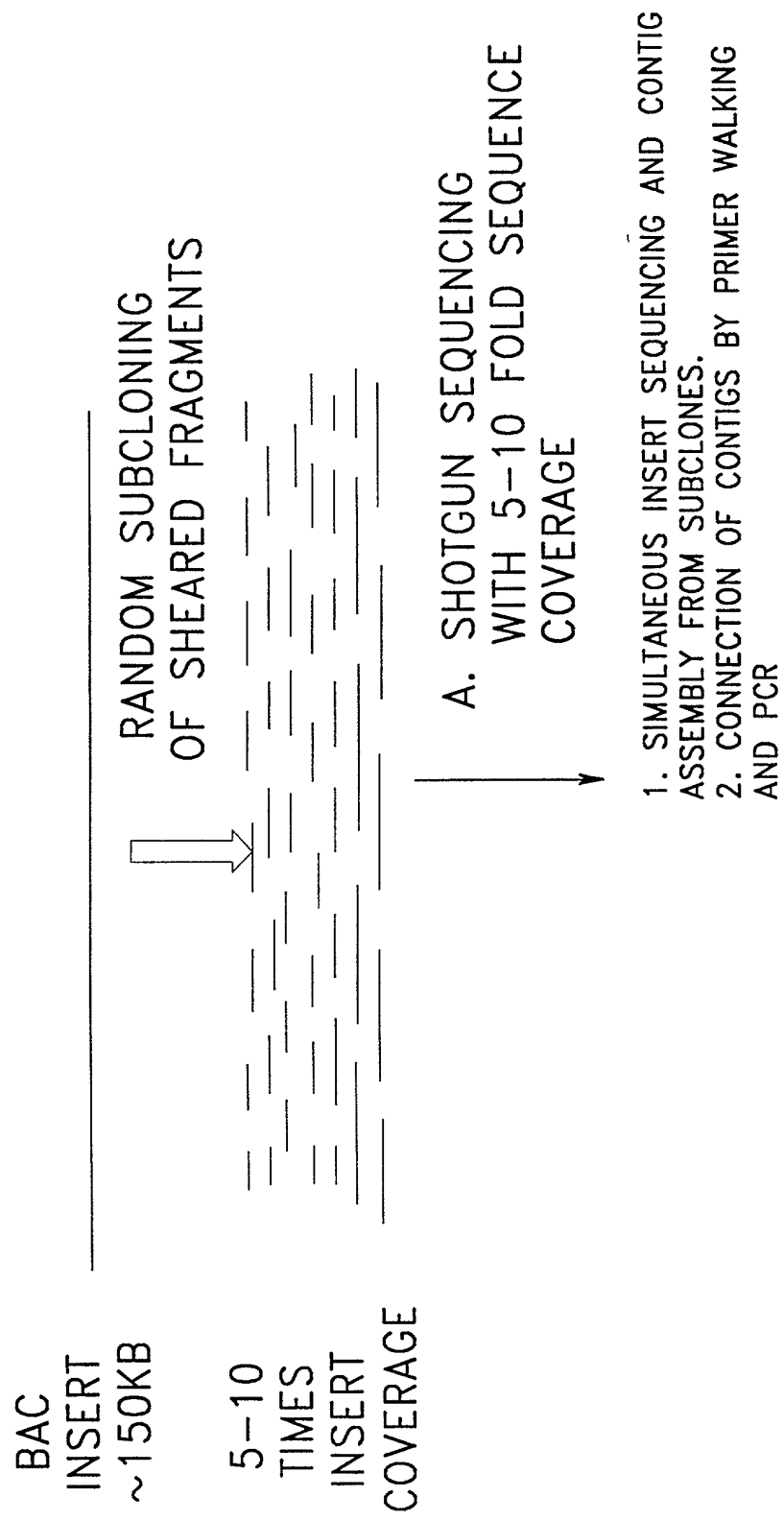


FIG.8

# STRATEGIES FOR SEQUENCING OF LARGE DNA FRAGMENTS

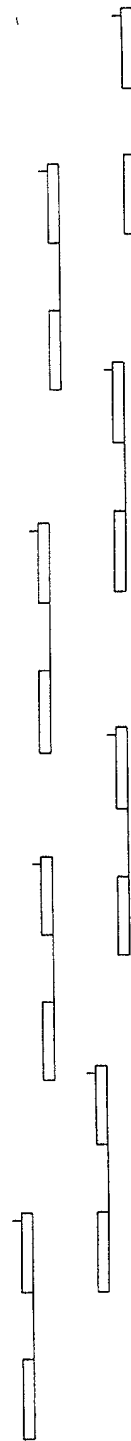


**FIG.9A**



## B. ORDERED SHOTGUN SEQUENCING-OSS

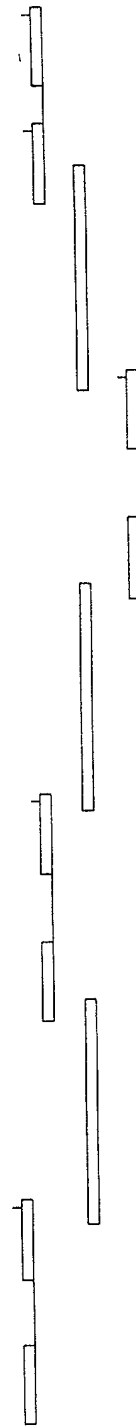
1. SIMULTANEOUS SEQUENCING OF BOTH ENDS OF LIMITED NUMBER OF SUBCLONES(1.5-2 FOLD SEQUENCE COVERAGE).
2. ASSEMBLY OF MINIMAL TILING PATH OF SUBCLONES BY PAIRWISE SEQUENCE OVERLAP.
3. PRIMER WALKING FOR EXTENSIVE SEQUENCING OF MINIMAL TILING PATH SUBCLONES



*FIG. 9B*

## C. MULTIPLE NUCLEATION POINT WALKING STRATEGY

1. SIMULTANEOUS COMPLETE SEQUENCING OF LIMITED NUMBER OF LARGE INSERT SIZE SUBCLONES WITH PAIRWISE END SEQUENCING FOR THE REST OF THEM RESULTS IN MINIMAL TILING PATH CONTAINING NUCLEATION POINTS OF HIGH QUALITY SEQUENCE.
2. TRANSPOSON MEDIATED SEQUENCING OF MINIMAL TILING PATH.



**FIG.9C**

0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800  
 29023,2 45244,2 58801,3 68316,9 75206,4 79971,4 83504,1 85720,1 87923,1 88876,5 89630 90447,7 91191,6 91627,5 91925,6 92286,5  
 0 65553,8 83342,9 90466 93252,9 94234,1 94791,3 95127,1 95519,8 95770,1 96043,3 96178,7 96361,7 96443,5 96591,5

PAIRWISE ONLY	29023,2	45244,2	58801,3	68316,9	75206,4	79971,4	83504,1	85720,1	87923,1	88876,5	89630	90447,7	91191,6	91627,5	91925,6	92286,5
MULTIPLE NUCLEATION	0	0	65553,8	83342,9	90466	93252,9	94234,1	94791,3	95127,1	95519,8	95770,1	96043,3	96178,7	96361,7	96443,5	96591,5
POINT																

# THE MAXIMUM SCAFFOLD LENGTH

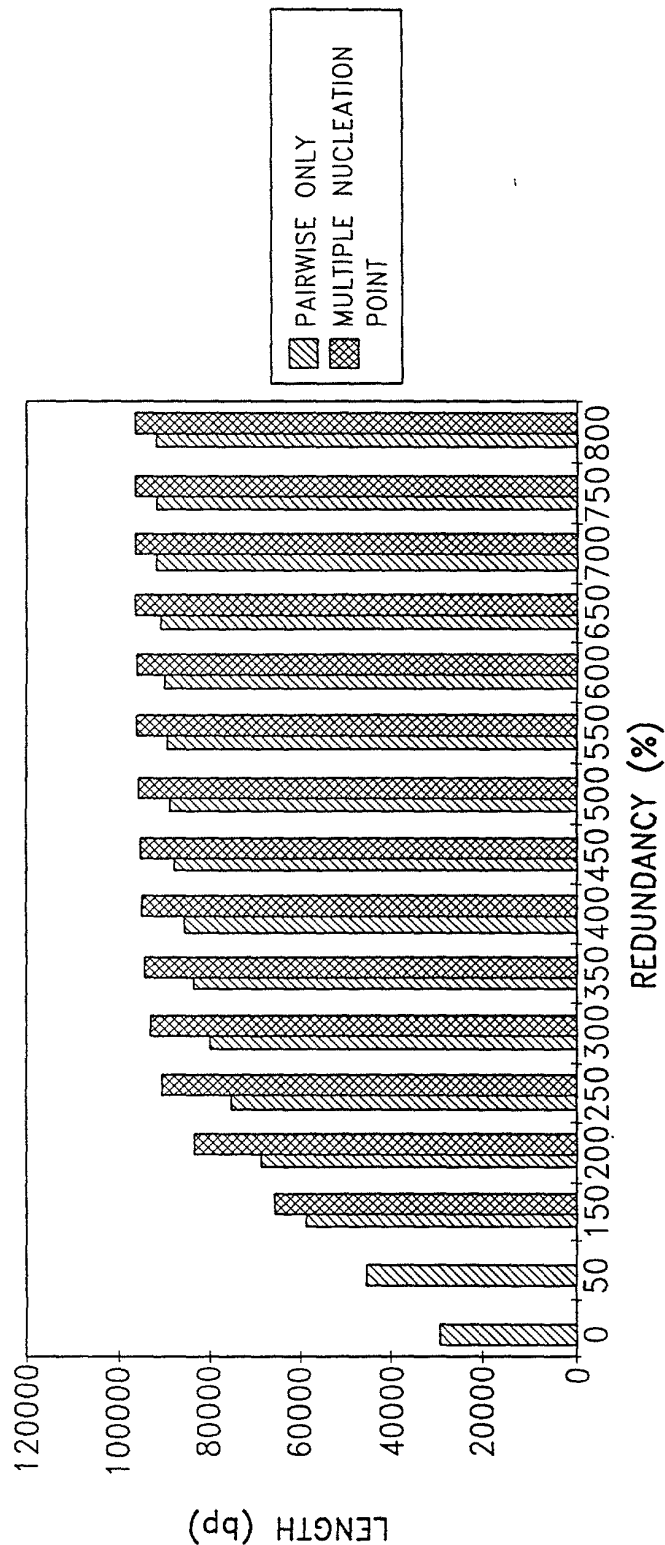


FIG. 10

PAIRWISE ONLY	8,908	16,455	17,729	15,476	12,483	9,701	7,522	6,11	4,974	4,208	3,662	3,24	2,894	2,655	2,467	2,274	2,074
MULTIPLE NUCLEATION POINT	0	0	2,5	2,338	2,185	2,046	1,931	1,808	1,676	1,577	1,484	1,43	1,374	1,332	1,302	1,264	1,264

# THE SCAFFOLDS NUMBER

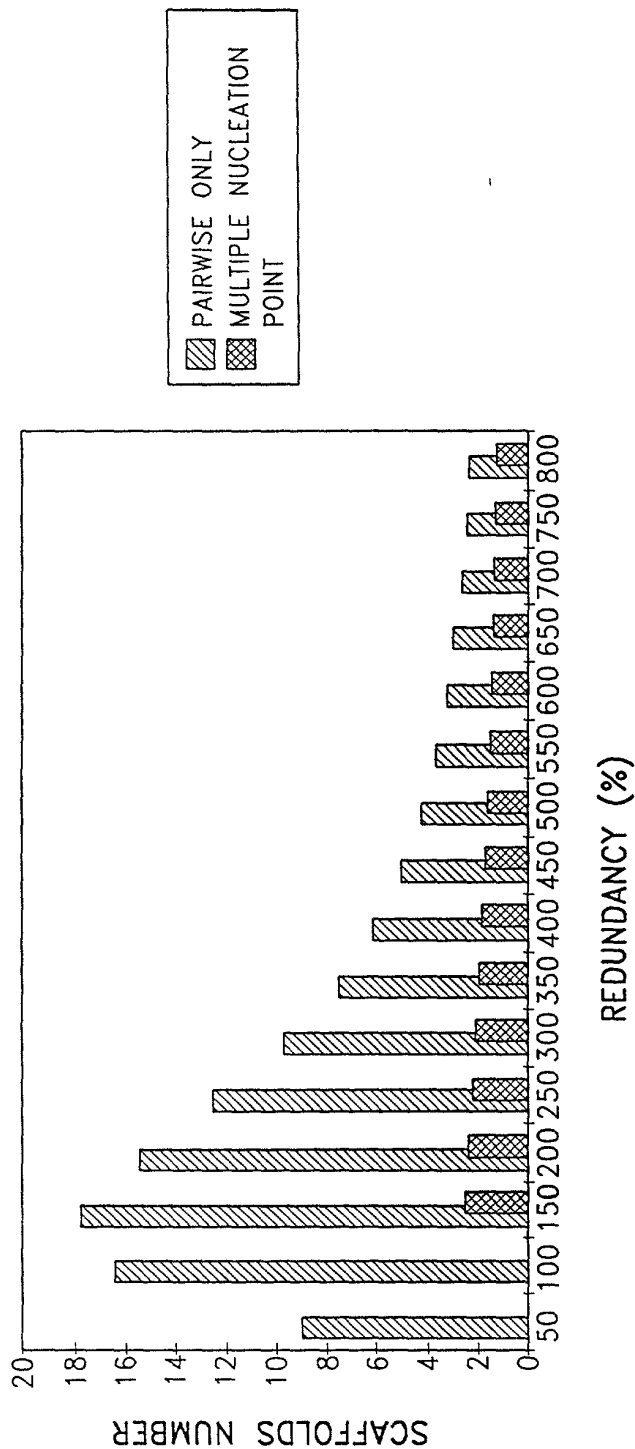


FIG. 11

PAIRWISE ONLY	2,02868	2,64877	3,13585	3,67497	3,77673	3,72902	3,33939	3,00032	2,60141	2,31273	2,12644	1,92935	1,76033	1,64194	1,53327	1,41807
MULTIPLE NUCLEATION	0	0	1,20333	1,59241	1,65915	1,63765	1,53826	1,42167	1,30039	1,16708	1,01968	0,94504	0,83793	0,78088	0,74216	0,66806

STANDARD DEVIATION OF THE SCAFFOLDS NUMBER

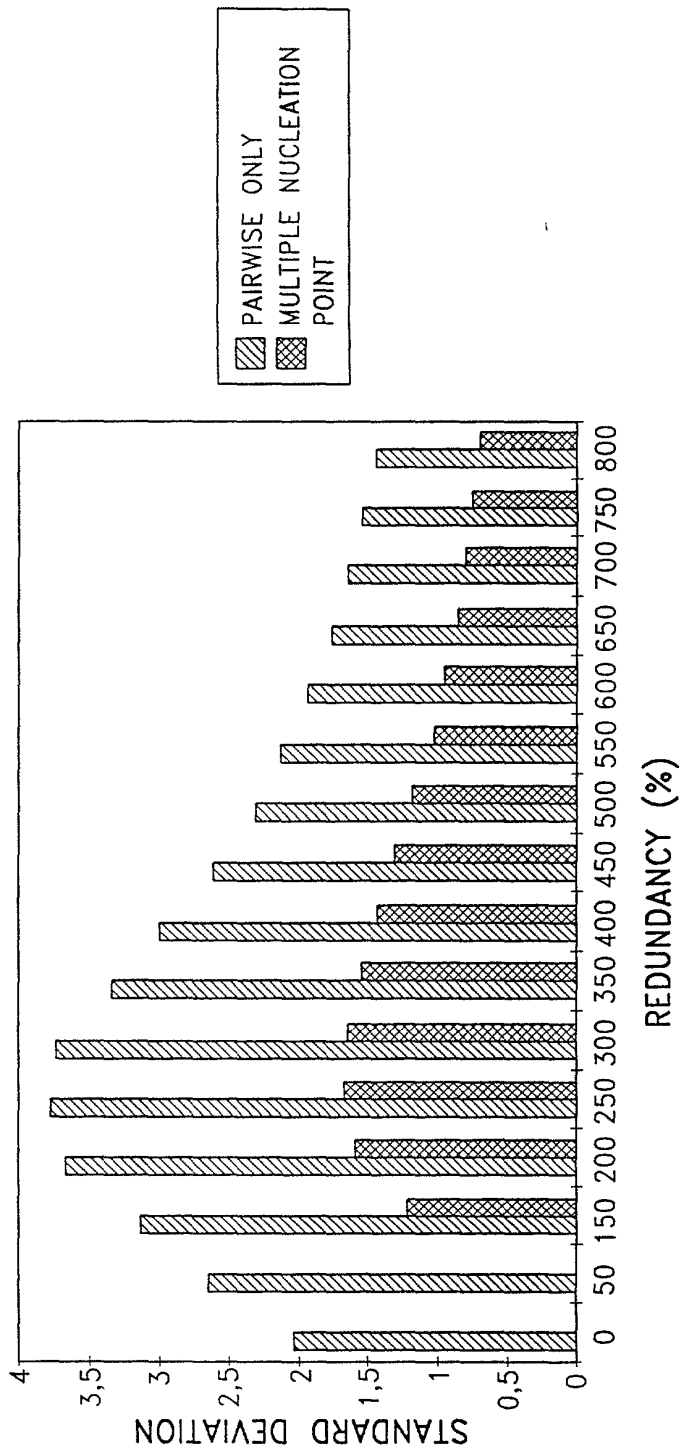
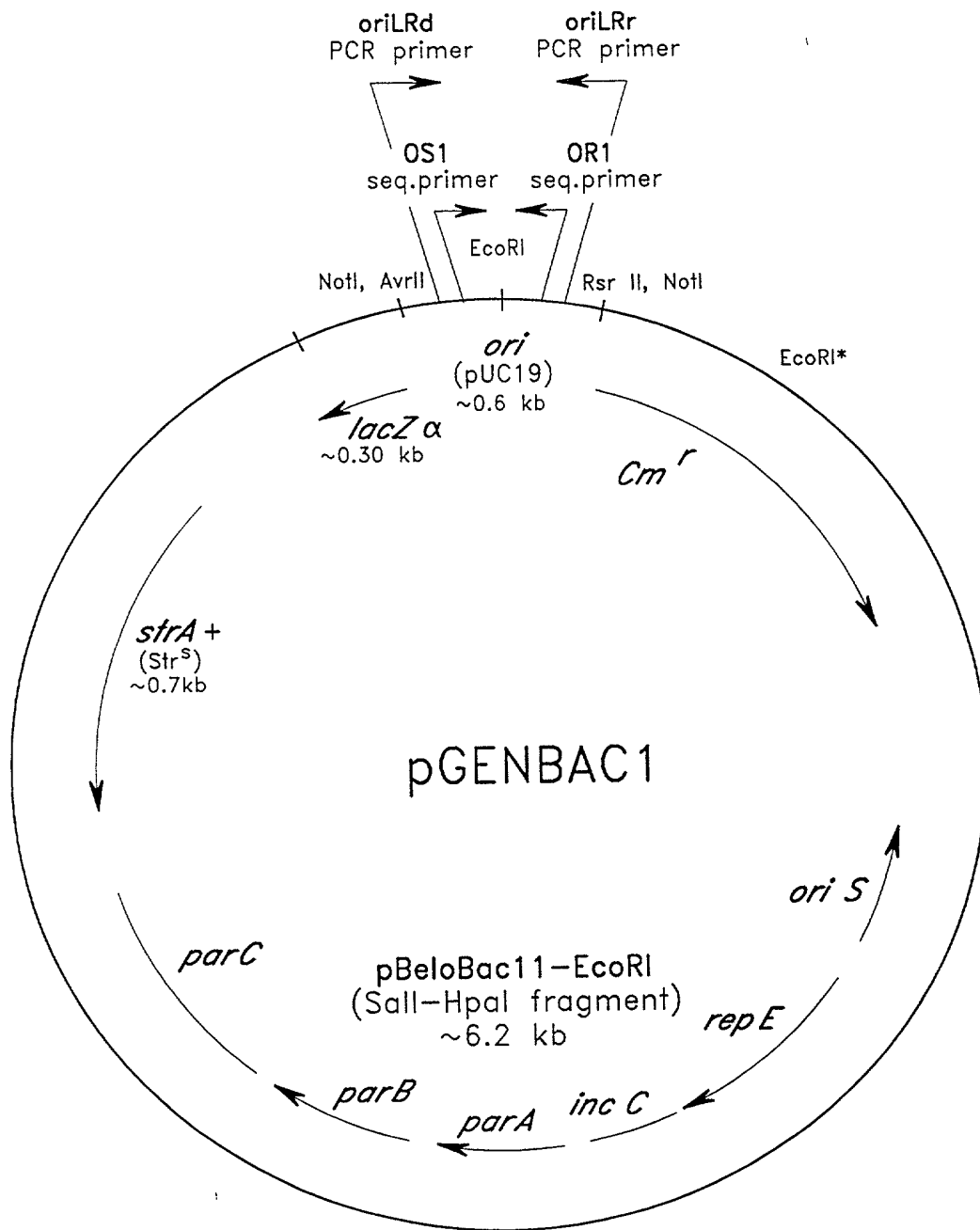
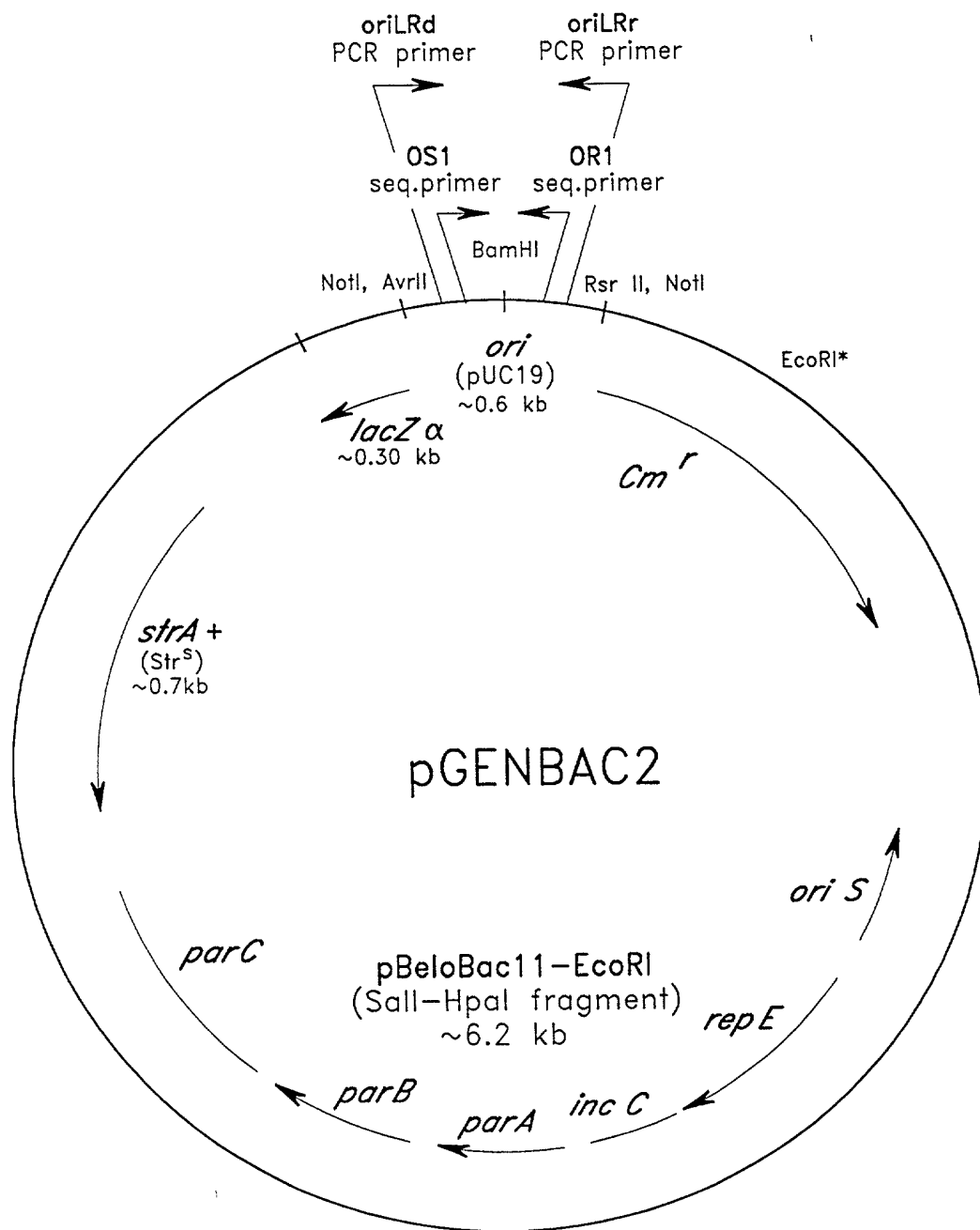


FIG. 12



**FIG. 13**



**FIG. 14**